Remarks

Applicants wish to thank the Examiners for the many courtesies extended at the telephonic interview held on Thursday, October 10, 2002 regarding the above-identified application.

The specification has been amended to remove reference to the URL, www.ncbi.nlm.nih.gov/BLAST/, in each of the following three paragraphs: paragraph at page 7, lines 1-28; paragraph beginning at page 11, line 26, and continuing through page 12, line 2; and paragraph beginning at page 16, line 27, and continuing through page 17, line 6.

The specification has been amended at page 19, lines 3-34, to correct a clerical error. The length of the 5' untranslated region cDNA of SEQ ID NO:1 should be given as 73 instead of 74. The ATG start codon of the cDNA shown in SEQ ID NO:1 is at nucleotides 74-76, hence, the untranslated leader region contains 73 nucleotides, and the translated protein-coding region begins at nucleotide 74. Thus, no new matter has been added.

The specification has been amended at page 20, lines 21-33, to correct four clerical errors. Firstly, the cDNA sequence in SEQ ID NO:3 perfectly matches the genomic sequence in SEQ ID NO:2 from nucleotide 1 to 59. The nucleotide sequence from position 1 to 59 of SEQ ID NO:3 corresponds to the nucleotide sequence from position 1497 to 1555 of SEQ ID NO:2. It is clear from page 20 of the specification at lines 21-33 that the region from nucleotide 60 to 249 (instead of nucleotide 60 to 250) of the cDNA perfectly matches the 190 bp (instead of 191 bp) region at the 3' end of the genomic DNA. The genomic DNA of SEQ ID NO:2 ends with a C nucleotide at position 2336; this C nucleotide corresponds to the C nucleotide in SEQ ID NO:3 at position 249, not position 250. Consequently, the region defined by nucleotides 60 to 249 is 190 bp, not 191 bp. Thus, no new matter is added.

Secondly, the specification has been amended at page 21, line 1, to correct a clerical error in the presentation of the nucleotide sequence of SEQ ID NO:4. On page 21, line 1 of the specification, SEQ ID NO:4 is given as the following:

CATGCCATGGCTTTATACTTCAAAAACTGCAC (SEQ ID NO:4)

The CT dinucleotide at position 11-12 of SEQ ID NO:4, indicated above by underlining, should be deleted. The above PCR primer, SEQ ID NO:4, was used to produce the sequence shown in SEQ ID NO:6 (the SAMS promoter and translation leader region with intron), and is complementary to the 3' end of SEQ ID NO:6. The SEQ ID NO:4 primer should have indicated a **replacement** of the wild-type soy CT

dinucleotide (underlined above) with the artificial GG dinucleotide, to create an NcoI restriction site that includes the ATG start codon. Thus, SEQ ID NO:4 on page 21, line 1 should not have had the wild-type CT dinucleotide. The basis for this clarification can be found in the specification beginning on page 21 at line 34:

> A 1305 bp SAMS genomic DNA fragment starting at nucleotide 856 and ending at nucleotide 2160 of SEQ ID NO:2: was amplified by PCR from the 2.5 kb ClaI clone.

If SEQ ID NO:4 is clarified to remove the CT dinucleotide, then the contiguous SAMS genomic sequence that is PCR amplified using the SEQ ID NO:4 primer should end, as indicated, at nucleotide 2160 of SEQ ID NO:2. It should also be noted that SEQ ID NO:4 is complementary to the sequence set forth in SEQ ID NO:2, hence, the 5'-CT of SEQ ID NO:4 corresponds to the complementary 5'-AG of nucleotides 2161-2162 of SEQ ID NO:2.

An amended Sequence Listing is also attached, in which SEQ ID NO:4 has been amended to delete the CT dinucleotide at positions 11-12, as described above. The following other corrections also appear in the amended Sequence Listing: 1) the serial number of this US patent application and the filing date appear in lines <140> and <141>, respectively; 2) a definition of the "unsure" nucleotides for three positions of SEQ ID NO:3 are given in <223> lines; and 3) the position and definition of an "unsure" nucleotide is given for each of SEQ ID NOs:17, 18, 19 and 20.

Claims 1-11 have been rewritten as claims 12-13 and 15-23. Claim 14 concerns an expression construct. Support for this can be found in the specification on page 10 starting on line 33 through line 5 on page 11. Thus, no new matter has been added. Furthermore, hybridization conditions are set forth in Example 3 on pages 17-18.

Claims 1-11 were rejected under 35 USC §112, first paragraph, on the ground that the "specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims, for the reasons of record set forth in the office action mailed July 5, 2001."

It is respectfully submitted that the claims as rewritten are believed to address the concerns raised with respect to 35 USC §112, first paragraph. Claim 12 (formerly claim 1) now recites an isolated nucleic acid fragment having constitutive promoter activity selected from the group consisting of :

a) an isolated nucleic acid fragment comprising the nucleic acid sequence of SEQ ID NO:6 or SEQ ID NO:14 or a subfragment thereof having constitutive promoter activity; and

b) an isolated nucleic acid fragment which can hybridize with any of the nucleotide sequences set forth in SEQ ID NO:6 or SEQ ID NO:14 under stringent conditions.

SEQ ID NO:6 is a subfragment of SEQ ID NO:14. SEQ ID NO:14 has promoter activity as evidenced by the data set forth in the declaration of Dr. Zhongsen Li which show that this larger fragment was able to drive the expression of two reporter genes, GUS and the SU resistant mutant ALS gene. The fragment having promoter activity that was used by Dr. Zhongsen Li as described in Dr. LI's declaration was substantially identical to SEQ ID NO:14, the difference being that the AG dinucleotide at the 3' end of SEQ ID NO:14, at positions 2161-2162, was changed to CC, in order to create an NcoI restriction site that encompasses the ATG start codon. This modification was done to facilitate cloning of different heterologous protein-coding sequences behind the longer SAMS promoter fragment. The substantially identical version of SEQ ID NO:14 was created by use of the 3' region of SEQ ID NO:6, which contains this artificially created NcoI site at the 3' end.

In view of the foregoing, it is respectfully submitted that the claims are now in form for allowance which allowance is respectfully solicited.

A petition for a two (2) month extension of time, a Version with Markings to Show Changes Made and a corrected sequence listing accompany this response.

Please charge any fees associated with the filing of this response to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company). If the fee is insufficient or incorrect, please charge or credit the balance to the above-identified deposit account.

Respectfully submitted,

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Version with Markings to Show Changes Made

In showing the changes, deleted material is shown in bold brackets and inserted material is shown as underlined.

In the Specification

Please amend the specification, paragraph at page 7, lines 1-28, as follows:

Moreover, the skilled artisan recognizes that substantially similar nucleic acid sequences encompassed by this invention are also defined by their ability to hybridize, under moderately stringent conditions (for example, 0.5 X SSC, 0.1% SDS, 60° C) with the sequences exemplified herein, or to any portion of the nucleotide sequences reported herein and which are functionally equivalent to the promoter of the invention. Preferred substantially similar nucleic acid sequences encompassed by this invention are those sequences that are 80% identical to the nucleic acid fragments reported herein or which are 80% identical to any portion of the nucleotide sequences reported herein. More preferred are nucleic acid fragments which are 90% identical to the nucleic acid sequences reported herein, or which are 90% identical to any portion of the nucleotide sequences reported herein. Most preferred are nucleic acid fragments which are 95% identical to the nucleic acid sequences reported herein, or which are 95% identical to any portion of the nucleotide sequences reported herein. Sequence alignments and percent similarity calculations may be determined using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences are performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments and calculation of percent identiy of protein sequences using the Clustal method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are GAP PENALTY=10, GAP LENGTH PENALTY=10, KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. A "substantial portion" of an amino acid or nucleotide sequence comprises enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to afford putative identification of that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Altschul, S. F., et al., (1993) *J. Mol. Biol. 215*:403-410) and Gapped Blast (Altschul, S. F. et al., (1997) *Nucleic Acids Res. 25*:3389-3402)[; see also www.ncbi.nlm.nih.gov/BLAST/)].

Please amend the specification, the paragraph beginning at page 11, line 26, and continuing through page 12, line 2, as follows:

Northern-blot hybridization experiments indicated that SAMS gene transcripts are present in a variety of soybean tissues and that the abundance of SAMS gene transcripts does not differ greatly from tissue to tissue (Figure 9 and Example 3). Strong expression of the SAMS gene was also inferred by the high frequency of occurrences of cDNA sequences with homology to SAMS (ESTs) in a soybean cDNA sequence database created by sequencing random cDNAs from libraries prepared from many different soybean tissues. ESTs encoding SAMS can be easily identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) J. Mol. Biol. 215:403-410[; see also www.ncbi.nlm.nih.gov/BLAST/]) searches for similarity to sequences contained in the BLAST "nr" database, *e.g.,* SAMS from Oryza sativa (EMBL Accession No. Z26867) or SEQ ID NO:1 provided herein. SAMS homologs were among the most abundant classes of cDNAs found in the soybean libraries. This indicated that SAMS was a highly expressed gene in most soybean cell types. The data obtained from sequencing many SAMS ESTs also indicated that there were several SAMS isoforms encoded by the soybean genome.

Please amend the specification, the paragraph beginning at page 16, line 27, and continuing through page 17, line 6, as follows:

ESTs encoding SAMS were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., *et al.,* (1993) *J. Mol. Biol. 215*:403-410[; see also www.ncbi.nlm.nih.gov/BLAST/]) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish, W. and States, D. J. (1993) *Nature Genetics 3*:266-272 and Altschul, S. F., *et al.* (1997) *Nucleic Acids Res. 25*:3389-3402) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly,

the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

Please amend the specification, paragraph at page 19, lines 3-34, as follows:

The soybean full length SAMS cDNA (SEQ ID NO:1), obtained in Example 2, was used to generate a probe to isolate a SAMS promoter. The full length SAMS cDNA sequence consisted of 1518 bp, and it had a 73**[4]** bp 5'-untranslated region and a PstI site at position 296. Because the cDNA clone was harbored in a pBluescript™ SK vector having a PstI site upstream of the EcoRI cloning site, digestion of the clone with Pst1 generated a 315 bp fragment of DNA. The resulting restriction fragment contained 19 bp of vector and cloning linker adapter sequence in addition to the 296 bp of SAMS cDNA sequence. This PstI fragment was labeled with α-^{32}P-dCTP, as described in Example 3, and used as a probe to screen a soybean genomic DNA library that had been constructed in a EMBL3 SP6/T7 vector (ClonTech, Palo Alto, CA). The library was plated with LE392 (ClonTech) cells at 50,000 plaque forming units (pfu) per 150 mm NZCYM agar plate (GIBCO BRL). Plaques were transferred to Hybond nylon membranes, and the plaque replicas were then denatured and neutralized according to the manufacturer (Amersham Life Science, Arlington Heights, IL). The phage DNA was fixed on the membranes by UV-crosslinking (Stratagene). After prehybridization at 65° for 1 hour in 0.5 M $NaHPO_4$, pH 7.2, 1 mM EDTA, 1% crystalline BSA (Sigma), and 7% SDS, the SAMS 315 bp Pst1 fragment probe was denatured in boiling water bath for 5 minutes and added to the same hybridization solution, and was hybridized at 65° for 24 hours. The membranes were washed in 40 mM $NaHPO_4$, pH 7.2, 1 mM EDTA, 0.5% crystalline BSA, and 5% SDS for 10 minutes at room temperature, and then 3x 10 minutes at 65° in 40 mM $NaHPO_4$, pH 7.2, 1 mM EDTA, and 1% SDS. The membranes were exposed to Kodak X-ray film (Sigma) at -80°. Positive SAMS genomic DNA phage clones were suspended in SM buffer, 50 mM Tris-Cl, pH 7.5, 100 mM NaCl, 0.2% $MgSO_4 \cdot 7H_2O$, and 0.1% gelatin, and purified by a secondary screening following the same procedure. Twenty three strongly hybridizing plaques were identified by the first screening from a total of $3x10^5$ pfu, and fifteen were later purified. DNAs were prepared from two of the purified phage clones (Ausubel et al., (1990) pp. 1.13.4-1.13.8), they were digested with BamHI, ClaI, PstI, and NcoI and prepared for a Southern blot. The blot was hybridized with the SAMS 315 bp PstI fragment probe prepared and used as above. A single positive fragment of clone 1 was identified from the ClaI digestion. Since the ClaI restriction site in the cDNA clone is 843 bp from the 5' end of the full length cDNA, the 2.5 kb ClaI fragment was expected to include about 1.7 kb of DNA upstream of the coding sequence, which was considered sufficient to contain the SAMS promoter.

Please amend the specification, paragraph at page 20, lines 21-33, as follows:

The cDNA sequence in SEQ ID NO:3 perfectly matches the genomic sequence in SEQ ID NO:2 from nucleotide 1 [2] to 59 [60] of the cDNA. There follows a region of 591 nucleotides in the genomic DNA that is absent from the cDNA. Then the region from nucleotide 60 to 249 [250] of the cDNA perfectly matches the 190 [191] bp region at the 3' end of the genomic DNA. This indicates the presence of a 591 nucleotide intron in the genomic DNA in the 5' transcribed, but untranslated, region of the SAMS gene. The presence of consensus 5' and 3' splice junctions in the genomic DNA at the exon-intron junctions supports this conclusion. Thus, the 53 bp at the 5' end of the cDNA used as the probe (SEQ ID NO:1) did not match the genomic sequence because the genomic sequence at that position in the alignment was from the intron. However, the 53 bp at the 5' end of the cDNA of SEQ ID NO:1 is very similar to the 60 nucleotides at the 5' end of the cDNA of SEQ ID NO:3, suggesting that the gene from which SEQ ID NO:1 was transcribed also contains an intron at the analogous position.

Please amend the specification, paragraph beginning at page 20, line 34, and continuing through page 21, line 9, as follows:

A 1305 bp SAMS genomic DNA fragment starting at nucleotide 856 and ending at nucleotide 2160 of SEQ ID NO:2: was amplified by PCR from the 2.5 kb ClaI clone. The promoter fragment was amplified from this fragment using primers sam-5 (SEQ ID NO:4) and sam-6 (SEQ ID NO:5) and Pfu DNA polymerase (Stratagene).

CATGCCATGG[CT]TTATACTTCAAAAACTGCAC (SEQ ID NO:4)

GCTCTAGATCAAACTCACATCCAA (SEQ ID NO:5)

An XbaI site and an NcoI site were introduced to the 5' end and 3' end, respectively, of the PCR fragment by using these specifically designed primers. The NcoI site includes the ATG start codon of the SAMS coding region. The resulting 1314 bp fragment is shown in SEQ ID NO:6 and includes the SAMS promoter and the translation leader region, which is interrupted by the 591 nucleotide intron.

In the Claims

Kindly delete claims 1-11 and submit the following new claims:

12. (new) An isolated nucleic acid fragment having constitutive promoter activity selected from the group consisting of :

a) an isolated nucleic acid fragment comprising the nucleic acid sequence of SEQ ID NO:6 or SEQ ID NO:14 or a subfragment thereof having constitutive promoter activity; and

b) an isolated nucleic acid fragment which can hybridize with any of the nucleotide sequences set forth in SEQ ID NO:6 or SEQ ID NO:14 under stringent conditions.

13. (new) A chimeric gene comprising at least one heterologous nucleic acid fragment operably linked to the isolated nucleic acid fragment of claim 1.

14. (new) An expression construct comprising the chimeric gene of claim 13.

15. (new) A plant comprising the chimeric gene of claim 13.

16. (new) The plant of claim 15 wherein said plant is a monocot selected from the group consisting of corn, rice, wheat, barley and palm.

17. (new) The plant of Claim 16 wherein said plant is a dicot selected from the group consisting of *Arabidopsis*, soybean, oilseed *Brassica*, peanut, sunflower, safflower, cotton, tobacco, tomato, potato, and cocoa.

18. (new) The plant of claim 17 wherein said plant is soybean.

19. (new) Seed of the plant as in any one of Claims 15, 16, 17 or 18 wherein said seed comprises in its genome the chimeric gene of claim 13.

20. (new) A method of increasing or decreasing the expression of at least one heterologous nucleic acid fragment in a plant cell which comprises:

(a)transforming a plant cell with the chimeric gene of Claim 13;

(b)growing at least one fertile mature plants from the transformed plant cell of step (a);

(c)selecting at least one plant containing a transformed plant cell wherein the expression of the heterologous nucleic acid fragment is increased or decreased.

21. (new) The method of Claim 20 wherein the plant is a monocot selected from the group consisting of corn, rice, wheat, barley and palm.

22. (new) The method of Claim 21 wherein the plant is a dicot selected from the group consisting *of Arabidopsis*, soybean, oilseed *Brassica*, peanut, sunflower, safflower, cotton, tobacco, tomato, potato, and cocoa.

23. (new) The method of Claim 22 wherein the plant is soybean.